

GENETIC CONTROL OF THE ANTIBODY RESPONSE TO TYPE III PNEUMOCOCCAL POLYSACCHARIDE IN MICE

II. RELATIONSHIP BETWEEN IgM IMMUNOGLOBULIN LEVELS AND THE ABILITY TO GIVE AN IgM ANTIBODY RESPONSE

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In the preceding paper of this series, we reported that CBA/HN mice (C mice), a genetically distinct subline of CBA mice, produce an extremely low, if any, IgM antibody response to Type III pneumococcal polysaccharide (SSS-III)¹ and to other polysaccharide antigens (1). Evidence was also presented to indicate that such mice lack an X-linked gene that governs responsiveness to SSS-III in a decisive manner (1). Since the X chromosome of man has been reported to carry genes that influence IgM immunoglobulin levels (2-4), and since several immune deficiency diseases appear to be X-linked (5-8), this study was undertaken to determine whether the failure of C mice to respond to SSS-III can be attributed largely to an impairment in their ability to synthesize IgM immunoglobulin. Serum IgM levels were studied in detail because mice immunized with SSS-III produce antibodies mostly of this immunoglobulin class. The results obtained show that C mice are unable to make a normal IgM antibody response to polysaccharide antigens, despite the fact that they possess the capacity to synthesize normal amounts of IgM immunoglobulin if antigen specific stimulation is bypassed.

Materials and Methods

Antigens and Immunization Procedures.—The immunological properties of the Type III pneumococcal polysaccharide (SSS-III) used have been described (9-12). Mice were given a single intraperitoneal injection of an optimally immunogenic dose (0.5 μ g) of SSS-III in 0.5 ml saline. The magnitude of the antibody response produced was assessed 5 days later.

Escherichia coli 0127 (lot 478203), *Salmonella typhosa* 0901 (lot 578448), *Salmonella typhimurium* (lot 579433), and *Serratia marcescens* (lot 577446) lipopolysaccharides (LPS) were

¹ *Abbreviations used in this paper:* B mice, BALB/cAnN; C mice, CBA/HN mice; LPS, lipopolysaccharides; PFC, plaque-forming cells; SSS-III, Type III pneumococcal polysaccharide.

purchased from Difco Laboratories, Detroit, Mich. Mice were given either one or a series of six intraperitoneal injections (two injections a week for 3 wk) of 10 μ g of LPS in 0.5 ml saline. Serum samples were collected 5 days after the last injection of LPS, and assayed for their hemolytic (IgM) antibody content.

Immunological Methods.—The procedures used for the detection of serum hemolytic (IgM) antibody and plaque-forming cells (PFC) specific for SSS-III or LPS has been described (1). Serum antibody titers were expressed as $1/\log_2$ of the highest dilution of serum giving complete hemolysis of a standard suspension of antigen-coated sheep erythrocytes (1); values for the PFC response were expressed as PFC/spleen or PFC/ 10^6 nucleated spleen cells (1).

Serum IgM immunoglobulin levels (mg IgM/ml serum) were determined by a modification of the technique of single radial diffusion (13, 14) as described by Biozzi et al. (15). The serum samples used for this purpose were the same as those employed for the detection of hemolytic (IgM) antibody in these or in previous studies (1), and were obtained 5 days after immunization with SSS-III or LPS. The results of preliminary studies affirmed that the IgM immunoglobulin levels of mice immunized with SSS-III were no greater than those of nonimmunized mice (unpublished observations).

Animals.—BALB/cAnN, CBA/HN mice, and progeny derived from crosses involving these two strains of mice were obtained from the Rodent and Rabbit Production Section of the National Institutes of Health (NIH), Bethesda, Md. CBA/CaJ mice were purchased from The Jackson Laboratories, Bar Harbor, Maine. All mice were 8–12 wk of age at the time of use.

Genetic Notation.—The notation of Gill et al. (16) was used to designate parental strains of mice, as well as F_1 , F_2 , and backcross generations. BALB/cAnN and CBA/HN mice were referred to as B mice and C mice, respectively. The first symbol used to describe a hybrid identifies the maternal member of a cross, e.g., CB mice are F_1 mice derived from a cross between female C mice and male B mice. For progeny derived from backcrosses, the symbol preceding the slash mark denotes the maternal member of the cross, e.g., CB/B mice are progeny derived from a backcross between female CB (F_1 mice) and male B mice. F_2 mice were referred to as either CB/CB or BC/BC mice.

The symbols X^+ and X^- were used to indicate the presence or absence of an X-linked gene that governs, in a decisive manner, the ability of mice to give an antibody response to SSS-III (1). Since this gene appears to be present in B (X^+X^+ , X^+Y) but not C (X^-X^- , X^-Y) mice (1), progeny derived from all crosses considered were classified accordingly with respect to their expected genotype.

Classification of Mice with Respect to Their Ability to Respond to SSS-III.—Mice were classified as low, intermediate, or high responders according to the magnitude of the PFC and serum antibody response produced 5 days after immunization with an optimally immunogenic dose (0.5 μ g) of SSS-III; the criteria used have been described in detail elsewhere (1). Briefly, C mice regularly give an extremely low mean PFC response ($<1,000$ PFC/spleen, <12 PFC/ 10^6 spleen cells) and no serum antibody can be detected ($1/\log_2 <1$); all mice giving a C type of antibody response were classified as low responders. In contrast, B mice produce the highest mean PFC and serum antibody response observed ($>13,000$ PFC/spleen, >70 PFC/ 10^6 spleen cells, and $1/\log_2 >7.0$); all mice giving a B type of antibody response were considered to be high responders. Mice giving an antibody response greater than that of C mice, but less than that of B mice, were classified as intermediate responders. The results of previous studies showed that for progeny derived from crosses between C and B mice, all X^+X^+ and X^+Y mice were high responders, all X^+X^- mice were intermediate responders, and all X^-X^- and X^-Y mice were low responders (1).

Statistics.—Student's t test (17) was used to evaluate the significance of the differences observed. Differences were considered to be significant when probability (P) values <0.05 were obtained.

RESULTS

Antibody Responses Produced by Mice Derived from Reciprocal Crosses.—The magnitude of the PFC and serum antibody response to an optimally immunogenic dose (0.5 μ g) of SSS-III was determined for BC and BC/BC mice, as well as for mice derived from backcrosses between BC mice and B or C mice; such mice constitute progeny of crosses that are the reciprocals of those considered earlier (1). With respect to the incidence of low responders obtained, the data of Table I were not only in accord with, but also confirmed, our original hypothesis (1), namely, that a major component involved in the antibody response to SSS-III is X-linked and appears to be present on the X chromosome of high responding B mice (X^+X^+ or X^+Y mice), but not low responding C mice (X^-X^- or X^-Y). Furthermore, these data also suggest that

TABLE I
Magnitude of the PFC and Serum Antibody Response Produced by Male and Female Progeny from Various Crosses

Mice	No. of Mice*	Hypothetical genotype	PFC/spleen		PFC/10 ⁶ spleen cells		1/log ₂ serum antibody titer	
			Range	Mean log ₁₀ \pm S \bar{x} †	Range	Mean log ₁₀ \pm S \bar{x} †	Range	Mean \pm S \bar{x}
BC	67/67	X^+X^-	500-14,400	3.62 \pm 6.04 (4179)	3-67	1.43 \pm 0.03 (27)	3-8	5.72 \pm 0.14
	81/81	X^+Y	1,000-26,750	3.73 \pm 0.03 (5371)	4-97	1.47 \pm 0.03 (30)	4-10	6.36 \pm 0.14
BC/BC	60/60	(X^+X^+ , X^+X^-)	1,050-31,950	3.78 \pm 0.04 (6080)	5-206	1.55 \pm 0.04 (36)	5-8	6.40 \pm 0.15
	28/60	X^+Y	1,500-25,900	3.82 \pm 0.06 (6645)	5-98	1.47 \pm 0.06 (29)	5-9	6.14 \pm 0.19
B/BC	32/60	X^-Y	<50-150	NC§	<0.5-1	NC	<1	NC
	23/23	X^+X^+	1,150-18,000	3.74 \pm 0.07 (5530)	6-77	1.44 \pm 0.07 (27)	5-8	6.61 \pm 0.16
	19/19	X^+Y	1,900-25,200	3.82 \pm 0.06 (6596)	12-194	1.58 \pm 0.06 (38)	5-8	6.16 \pm 0.22
BC/B	44/44	(X^+X^+ , X^+X^-)	350-24,000	3.82 \pm 0.05 (6592)	2-166	1.59 \pm 0.05 (39)	4-8	5.84 \pm 0.19
	15/27	X^+Y	5,950-19,200	4.01 \pm 0.04 (10,136)	34-108	1.78 \pm 0.04 (61)	4-7	5.40 \pm 0.47
C/BC	12/27	X^-Y	<50-300	NC	<0.5-3	NC	<1	NC
	15/15	X^+X^-	50-9,650	3.37 \pm 0.15 (2346)	0.3-62	1.21 \pm 0.15 (16)	4-7	5.20 \pm 0.26
BC/C	20/20	X^-Y	<50-200	NC	<0.5-2	NC	<1	NC
	10/18	X^-X^-	<50-100	NC	<0.5-1	NC	<1¶	NC
	8/18	X^+X^-	1,000-6,400	3.37 \pm 0.11 (2338)	6-37	1.16 \pm 0.10 (14)	4-6	4.50 \pm 0.27
	10/19	X^+Y	1,150-7,550	3.39 \pm 0.09 (2431)	6-40	1.19 \pm 0.10 (16)	4-6	5.50 \pm 0.22
	9/19	X^-Y	<50-50	NC	<0.5-0.6	NC	<1	NC

* No. of mice giving a stated response on the basis of genotype/total no. of mice examined.

† Geometric means are shown in parentheses.

§ Not calculable, since no PFC or serum antibody could be detected in all but a few of the mice examined.

|| No serum antibody detected in any of the mice examined.

¶ 1/log₂ serum antibody titer of 6 for one mouse.

additional factors, presumably autosomal genes, may act in a complex manner to influence the magnitude of the antibody response produced by mice having the same hypothetical genotype with respect to the X-linked gene. For example, among X⁺Y mice, the response produced by BC/B mice was similar to that of high responding B mice (1); however, BC, BC/BC, B/BC, and BC/C mice gave low to intermediate responses. The basis for such quantitative differences will be considered later in this paper in relation to the results obtained in other studies.

Responses Produced by Genotypically Identical Reciprocal F₁ Mice.—Since female BC and CB mice possess identical genotypes, these mice should have responded similarly to SSS-III; this was not the case. Instead, the magnitude of both the PFC and serum antibody responses of CB mice (Table II) was significantly greater (about two-fold) than that produced by BC mice ($P < 0.001$ for all three immunological parameters considered).

Serum IgM Levels of Mice Giving a High, Intermediate, or Low Antibody Response to SSS-III.—Serum IgM levels were determined for B and C mice, as well as for progeny derived from all of the crosses and backcrosses considered in this and the previous work (1). Almost all (>80%) of the serum samples collected during the course of these investigations were used for such analyses; consequently, the results obtained provide representative values for the magnitude, range and distribution of IgM levels for each group of mice examined. All samples used were identified with respect to the cross, or parental strains of mice, from which they were derived and were classified as to whether they were obtained from high, intermediate or low responders to SSS-III. Histograms, such as those illustrated in Fig. 1, were constructed for all groups of serum samples assayed in order to determine whether one or more discrete distributions of IgM levels were present within each group of mice.

During the entire course of this study, only two types or categories of IgM levels were encountered; both are depicted in Fig. 1 and were referred to as low IgM levels (0.10–0.35 mg IgM/ml) and high IgM levels (0.40–1.00 mg IgM/ml). Since almost all (about 95%) of the serum samples examined could

TABLE II
Differences Between Female BC and CB Mice in their Ability to Respond to an Optimally Immunogenic Dose (0.5 μg) of SSS-III

No. of mice	Hypothetical genotype	Mice	PFC/spleen	PFC/10 ⁶ spleen cells	1/log ₂ serum antibody titer
			<i>Mean log₁₀ ± S\bar{x}</i>		<i>Mean ± S\bar{x}</i>
67	X ⁺ X ⁻	BC	3.62 ± 0.04 (4,179)*	1.43 ± 0.03 (27)	5.72 ± 0.14
63	X ⁺ X ⁻	CB	3.90 ± 0.03 (7,940)	1.62 ± 0.03 (42)	6.75 ± 0.27

* Geometric means are shown in parentheses.

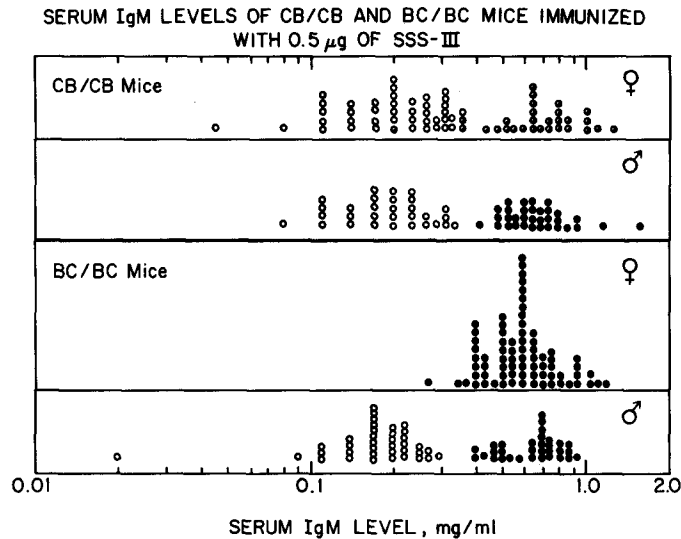


FIG. 1. IgM levels of male and female F_2 mice. Each point shown indicates the IgM level (mg/ml) of a single mouse. ○, low antibody response to SSS-III; ●, high antibody response to SSS-III; ◐, intermediate response to SSS-III.

be assigned, with no difficulty, to one of these two categories or distributions, histograms for the remaining groups of serum samples assayed are not shown; rather, only mean values for one or each of the two categories of IgM levels present are listed in Tables III and IV. The results obtained show that all low responders to SSS-III had low IgM levels. However, even though the antibody response to SSS-III is predominantly an IgM antibody response (9, 10, 18), both high and intermediate responders had high IgM levels of the same magnitude. Whereas the IgM levels of man have been reported to be influenced directly by an X-linked gene dose effect (2-4), the IgM levels of the mice examined in the present work appeared to be transmitted as an X-linked dominant trait. For example, X^+X^+ and X^+X^- mice differed with respect to gene dose for the X-linked component and responsiveness to SSS-III; yet, the IgM levels of these mice were almost identical.

Effect of Treatment with LPS on the Magnitude of the IgM Levels of B and C mice.—B and C were given one or more injections of 10 μ g of LPS derived from different bacterial species; such treatment has been shown to result in a substantial increase in IgM level (19). 5 days after the last injection, serum samples were collected and assayed for the presence of antibody specific for LPS, as well as for their IgM content.

Treatment with LPS resulted in about a four- to ninefold increase in the IgM levels of C mice (Table V); the resultant values obtained (0.60-1.24 mg/ml) were at least as great, or greater, than those of B mice not given LPS (0.50 mg/ml, Tables III and IV). More important, in spite of the increased amounts

TABLE III
Serum IgM Immunoglobulin Levels of Female Mice Derived from Various Crosses

No. of mice	Hypothetical genotype	Type of response to SSS-III	Mice	IgM immunoglobulin level	
				Geometric mean	Log ₁₀ mean ± S \bar{x}
				<i>mg/ml serum</i>	
21	X ⁺ X ⁺	High	B	0.500	-0.301 ± 0.030
23	"	Intermediate*	B/BC	0.568	-0.246 ± 0.028
33	X ⁺ X ⁻	Intermediate	B/CB	0.642	-0.192 ± 0.019
30	"	"	CB/CB	0.640	-0.194 ± 0.032
25	"	"	CB/C	0.740	-0.131 ± 0.018
42	"	"	BC	0.593	-0.227 ± 0.017
8	"	"	BC/C	0.602	-0.220 ± 0.058
46	"	"	CB	0.662	-0.179 ± 0.013
15	"	"	C/BC	0.488	-0.312 ± 0.034
40	X ⁻ X ⁻	Low	CB/CB	0.195	-0.709 ± 0.031
17	"	"	CB/C	0.113	-0.948 ± 0.046
29	"	"	C/CB	0.122	-0.915 ± 0.026
10	"	"	BC/C	0.053	-1.276 ± 0.125
10	"	"	C	0.140	-0.854 ± 0.030
44	(X ⁺ X ⁺ X ⁺ X ⁻)	(High + intermediate)‡	BC/B	0.451	-0.345 ± 0.017
74			BC/BC	0.584	-0.234 ± 0.015
26			CB/B	0.586	-0.232 ± 0.019

* These mice gave an intermediate PFC response and their serum antibody titers were lower than expected on the basis of their hypothetical genotype; consequently, they were classified as intermediate responders.

‡ Both high and intermediate responders were present; these could not be distinguished from each other.

of IgM immunoglobulin produced, no serum antibody, specific for *E. coli* LPS, could be detected (Table VI). Lesser increases (two- to threefold) in IgM immunoglobulin were noted for B mice given LPS (Table V) and although antibody specific for *E. coli* LPS could be detected (Table VI), there appeared to be no directed relationship between IgM levels and the amount of serum antibody found. Multiple injections of *E. coli* LPS resulted in still higher IgM levels (1.50 mg/ml); yet, the serum antibody titers obtained were lower than those of mice given only one injection of LPS.

Treatment with *S. typhosa*, *S. typhimurium*, and *S. marcescens* LPS likewise increased the IgM levels of both B and C mice (Table V); but neither B or C mice produced a measurable serum antibody response to these antigens (data not shown). It has not been established whether these preparations of LPS were

TABLE IV
Serum IgM Immunoglobulin Levels of Male Mice Derived from Various Crosses

No. of mice	Hypo- thetical genotype	Type of response to SSS-III	Mice	IgM immunoglobulin level	
				Geometric mean	Log ₁₀ mean ± S \bar{x}
				<i>mg/ml serum</i>	
37	X ⁺ Y	High	B	0.490	-0.310 ± 0.020
4	"	"	CB/B	0.647	-0.189 ± 0.023
15	"	"	BC/B	0.529	-0.276 ± 0.040
36	"	"	B/CB	0.597	-0.224 ± 0.016
34	"	"	CB/CB	0.670	-0.174 ± 0.020
26	"	"	CB/C	0.603	-0.219 ± 0.022
74	"	Intermediate*	BC	0.616	-0.210 ± 0.011
19	"	"	B/BC	0.525	-0.279 ± 0.033
32	"	"	BC/BC	0.635	-0.197 ± 0.019
9	"	"	BC/C	0.515	-0.288 ± 0.043
7	X ⁻ Y	Low	CB/B	0.218	-0.662 ± 0.058
12	"	"	BC/B	0.053	-1.276 ± 0.066
37	"	"	BC/BC	0.171	-0.766 ± 0.032
18	"	"	C/BC	0.049	-1.306 ± 0.075
20	"	"	CB/CB	0.189	-0.723 ± 0.029
26	"	"	CB/C	0.099	-1.003 ± 0.030
35	"	"	C/CB	0.101	-0.995 ± 0.030
9	"	"	BC/C	0.057	-1.247 ± 0.174
20	"	"	C	0.181	-0.743 ± 0.031
91	"	"	CB	0.142	-0.847 ± 0.014

* These mice gave an intermediate PFC response and their serum antibody titers were lower than expected on the basis of their hypothetical genotype; consequently, they were classified as intermediate responders.

weakly immunogenic for both of the strains of mice used, or whether they failed to function properly in the procedure used for the detection of antibody specific for LPS.

IgM Levels and SSS-III-Specific Antibody Response of CBA/CaJ Mice.— IgM levels, in addition to PFC and serum antibody responses to 0.5 μ g SSS-III, were determined for CBA/CaJ mice; the results of homograft and mixed lymphocyte culture studies affirmed that these mice are histocompatible with the low responding C mice used in this work (to be published). The data of Table VII show that, although the IgM levels of CBA/CaJ mice were virtually the same as those of high responding B mice (Tables II and III), CBA/CaJ mice gave a low to intermediate PFC and serum antibody response to SSS-III. These findings, in addition to those of Tables II and III, indicate that mice having high IgM levels do not necessarily give a high IgM antibody response to SSS-III.

TABLE V
Effect of Treatment with Different Types of LPS Preparations on the Serum IgM Immunoglobulin Levels of B and C Mice

Treatment			IgM immunoglobulin level	
LPS	Dose	No. injections	B mice	C mice
	$\mu\text{g}/\text{mouse}$		$\text{mg}/\text{ml serum}$	
<i>E. coli</i>	10	1	1.11*	0.60
			(0.043 \pm 0.033)‡	(-0.219 \pm 0.048)
	10	6§	1.50	0.936
			(0.176 \pm 0.019)	(-0.029 \pm 0.088)
<i>S. typhosa</i>	10	1	1.08	0.940
			(0.034 \pm 0.023)	(-0.027 \pm 0.024)
<i>S. typhimurium</i>	10	1	1.39	1.24
			(0.142 \pm 0.033)	(0.093 \pm 0.041)
<i>S. marcescens</i>	10	1	1.63	1.11
			(0.213 \pm 0.048)	(0.046 \pm 0.031)

* Geometric mean.

‡ $\text{Log}_{10} \pm S\bar{x}$ for 3-7 mice; all determinations were made on serum samples collected 5 days after the last injection of LPS.

§ Two injections a week for 3 wk.

DISCUSSION

Cooperation between "helper" T cells and bone marrow-derived precursors of antibody-forming cells (B cells) has not been found to be required for an antibody response to SSS-III, a linear polymer composed of identical epitopes (20-25). Consequently, the antibody response to this antigen is considered to be largely a B-cell response in which the antibody produced is predominantly of the IgM class (22, 24). Furthermore, the avidity of antibody specific for SSS-III remains essentially consistent over a 10,000-fold range of immunizing doses and after reimmunization with low or high doses of antigen, indicating the lack of an antigen-mediated cell selection process (26). These findings are consistent with the view that the antibody response to SSS-III is the result of the direct stimulation by antigen of a highly restricted population or clone of B cells.

The results of these and of previous studies (1, Table I) show that CBA/HN mice (C mice) lack an X-linked gene that plays a decisive role in determining responsiveness, not only to SSS-III, but also to other polysaccharide antigens that elicit primarily an IgM antibody response. Since several immunoglobulin deficiency diseases of man have been reported to be X-linked (5-8), one objective of this study was to determine whether the failure of C mice to respond to polysaccharide antigens was the result of a defect in their capacity to synthesize IgM immunoglobulin. The results obtained (Tables III and IV) showed that C mice, as well as all other mice lacking the X-linked gene, i.e. all X-X⁻ or X-Y

TABLE VI
Serum Hemolytic (IgM) Antibody Titers of B and C Mice Immunized with E. coli LPS

Immunization procedure		Serum antibody titer	
Dose	No. injections	B mice	C mice
μg			
10	1	$6.20 \pm 0.58^*$	None detected
10	6	3.29 ± 0.68	None detected

* $1/\log_2 \pm S\bar{x}$ for 5-8 mice; all determinations were made 5 days after the last injection of LPS.

TABLE VII
Serum IgM Immunoglobulin Levels, PFC, and Serum Antibody Responses of CBA/CaJ Mice Immunized with 0.5 μg of SSS-III

Sex	No. mice	IgM levels	Mean antibody response to SSS-III		
			PFC/spleen	PFC/ 10^6 spleen cells	Serum antibody
		<i>mg/ml serum</i>			
Male	8	$-0.25 \pm 0.04^*$ (0.56)	$3.54 \pm 0.128^*$ (3,491)	$1.387 \pm 0.138^*$ (24)	$5.58 \pm 0.02\ddagger$
Female	15	-0.26 ± 0.02 (0.55)	3.344 ± 0.089 (2,210)	1.132 ± 0.081 (14)	6.00 ± 0.00

* $\text{Log}_{10} \pm S\bar{x}$; geometric means in parentheses.

$\ddagger 1/\log_2 \pm S\bar{x}$.

mice, had IgM levels much lower than those of mice possessing this gene. Such low levels could reflect either an inability to respond to polysaccharide antigens encountered in the environment, or an inability to make IgM antibodies in response to most antigens. However, the fact that treatment with bacterial lipopolysaccharides (LPS) resulted in a substantial increase in the IgM levels of C mice (Table V) indicates that the biosynthetic machinery required for the production of IgM immunoglobulin is intact, and that the X-linked genetic defect observed is most likely related to the mechanism involved in triggering lymphocytes to differentiate following antigenic stimulation. In this context, it should be noted that LPS is considered to be a B-cell mitogen (27, 28) and may stimulate lymphocytes to produce IgM immunoglobulin by a process independent from that involving antigen-specific receptors; the fact that C mice produce large amounts of IgM immunoglobulin, but no specific antibody following treatment with LPS (Table VI), provides support for such a view.

All of the mice examined in this and the previous work (1) could be classified as being low, intermediate, or high responders to SSS-III; the magnitude of the antibody response produced after immunization with SSS-III was related directly to gene-dose for the X-linked component (1, Table I). However, no such relationship was observed with respect to IgM immunoglobulin levels. Although all low responders had low IgM levels, both high and intermediate

responders had high IgM levels of the same magnitude (Tables III and IV). Furthermore, CBA/CaJ mice had IgM levels, equal to those of high responding B mice; yet, these mice gave a low to intermediate antibody response to SSS-III (Table VII). These findings indicate that high IgM levels per se do not necessarily determine whether mice will give a high antibody response to SSS-III.

Despite the almost monoclonal characteristics of the antibody response to SSS-III, the genetic mechanisms that govern responsiveness to this antigen of relatively simple structure appear to be quite complex. They include an X-linked dominant gene that determines responsiveness to SSS-III in a decisive manner (1), in addition to other factors, presumably autosomal genes, that influence the magnitude of the response produced by mice possessing the X-linked gene (1, 29). The following observations, derived from these and other studies (1, Tables I and II), best illustrate some of the quantitative effects which appear to be produced by the latter.

First, the results of previous studies showed that for B mice immunized with SSS-III there was a direct relationship between the magnitude of the PFC response and the serum hemolytic antibody titer obtained (10, 11). However, among groups of hybrids having identical genotypes with respect to the X-linked gene, this relationship did not always exist. When the responses produced by such groups of mice were compared, significant differences in the magnitude of the PFC response frequently were not accompanied by significant differences in the magnitude of the serum antibody response and conversely. Second, high and intermediate responding mice, having identical genotypes with respect to the X-linked gene, may differ significantly in their ability to respond to SSS-III. This is most evident when one compares the results obtained with progeny derived from (a) backcrosses to C or B mice, and (b) backcrosses to BC or CB mice. In the first case, without exception, mice derived from backcrosses to C mice gave responses that were lower than those of mice derived from corresponding backcrosses to B mice. In the second case, for at least two of the three immunological parameters considered, mice derived from backcrosses to BC mice invariably gave lower responses than those produced by mice derived from backcrosses to CB mice. The above observations apply regardless of the genotype of the maternal or paternal member of the crosses considered. The results obtained with both types of F_1 mice examined in this study (Table II) further serve to illustrate some of the quantitative differences noted between groups of mice having identical genotypes and emphasize the need for conducting reciprocal crosses in all studies on the genetic control of the immune response to avoid making erroneous conclusions. Third, among all X^+Y mice examined, BC, BC/BC, B/BC, and, BC/C mice, as well as all female B/BC mice (X^+X^+) should have been high responders. Instead, these mice gave an intermediate PFC response and their serum antibody responses were lower than expected on the basis of their hypothetical genotype.

While the mechanisms responsible for producing these and all of the other quantitative effects considered above remain to be defined, they could be attributed to differences between C and B mice with respect to regulatory, rather than structural, gene functions. In this context, the existence of two functionally distinct types of thymus-derived cells (amplifier and suppressor T cells), that act in either a positive or a negative manner to influence the magnitude of the antibody response to SSS-III (24, 30-34), implies that these activities may be under separate genetic control and could act independently to regulate the number of antibody-forming cells obtained after immunization and the amount of antibody synthesized by such cells (1).

Although immunoglobulin levels and antibody responses other than IgM have thus far not been investigated in great detail, the results of preliminary studies indicate that (a) C and B mice do not have IgG and IgA levels which differ significantly, and that (b) both types of mice, hyperimmunized with ferritin, give good IgG antibody responses to this antigen (unpublished observations). Nevertheless, C mice provide an excellent experimental model for the study of X-linked immunologic deficiencies. While the genetic defect observed is less severe than that described in clinical situations (5-8), the present work provides some indications concerning the level of the immune response at which such a defect can occur and clearly shows that C mice possess cells fully capable of synthesizing IgM immunoglobulin in large quantity if specific antigenic stimulation is bypassed. Further studies are being conducted to obtain more precise information on the nature of this genetic defect which appears to involve a critical step in the process by which lymphocytes are triggered to differentiate in response to antigen.

SUMMARY

Serum IgM immunoglobulin levels and antibody responses to an optimally immunogenic dose of Type III pneumococcal polysaccharide (SSS-III) were assessed for F_1 , F_2 , and backcross progeny derived from crosses between high responding BALB/cAnN (B) and low responding CBA/HN (C) mice. The results obtained confirmed our original hypothesis, namely, that a major component, present on the X chromosome, governs the ability to respond to SSS-III in a decisive manner. Although all low responding C mice had low IgM levels, both intermediate and high responders had high IgM levels of the same magnitude. Treatment with bacterial lipopolysaccharides (LPS) resulted in a significant increase in the IgM levels of low responding C mice. While the IgM levels attained were similar to those of high responding B mice, not given LPS, no antibody specific for LPS appeared to be produced. These findings suggest that C mice are unable to make an IgM antibody response to SSS-III and other polysaccharide antigens, despite the fact that they possess the capacity to synthesize normal amounts of IgM immunoglobulin.

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